Direct Patterning of Silanized-Biomolecules on Semiconductor Surfaces

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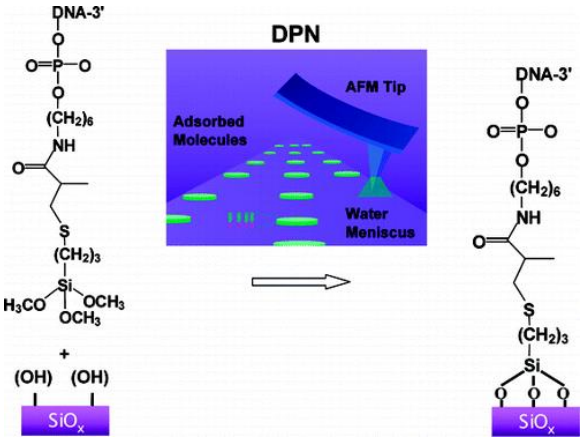
Abstract



A novel approach to pattern silanized-biomolecules directly onto glass (SiO_x) substrates via Dip-Pen nanolithography (DPN) and microcontact printing (μCP) is presented. Subsequent hybridization reactions of DPN patterned silanized-DNA with its complementary strands provide "proof-of-concept" that the patterned oligonucleotides maintain their biological activities. The fabrication strategy does not require premodification of substrates and offers a cheap and robust way to immobilize molecules on electronically important semiconductor surfaces.

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Introduction

Developing methods to pattern and immobilize biological molecules with micro-to-nanometer scale control has resulted in a broad range of new technological advancements not only in basic research but also in diagnostics and drug discovery.(1-4) However, many challenges remain, particularly the development of patterning methods that combine micro-to-nanoscale surface features with adhesion chemistries that not only provide selectivity in biomolecule binding and positioning but also preserve biological activities. Spatially controlled, directed biomolecule binding can be utilized as artificial surface receptors that will allow biological signaling processes to be studied and may prove to be useful in the design and development of detection systems or laboratories-on-a-chip.(5) Another potential application is the development of microelectromechanical systems (MEMS) or solar cells if predesigned, spatially controlled biomolecule arrays can be prepared on inert substrates and retain their biological function.(6-9)

With a few notable exceptions, the vast majority of predesigned, spatially controlled biomolecule arrays reported have been limited to gold substrates, which are not ideal for electronic or optical materials.(1, 3, 10, 11) When biological microarrays have been prepared on inert substrates such as Si/SiO_x, amino or thiosilanes function as the linker molecule, providing a positive charge, reactive group, or both for biomolecule attachment.(12, 13) Typically, the entire silicon/glass surface is modified with an aminosilane, followed by reaction with a cross-linking molecule such as *N*-hydroxysuccinimidyl ester or maleimide-terminated DNA.(14-16) Selective deposition of silane molecules has been challenging because of their propensity to polymerize before they can be deposited.(17-20) Additionally, deposited silane molecules have to be "cured" for them to bond covalently to the silicon surface.(21) To overcome this problem, self-assembled monolayers (SAMs) of alkyltrichlorosiloxanes have been widely studied and are typically prepared via microcontact printing (μCP) followed backfilling with a functionalized trimethoxysilane.(22, 23) Alternatively, in an elegant study, Mirkin et

al.(12) successfully utilized Dip-Pen nanolithography (DPN) to deliver DNA onto premodified silicon surfaces where the entire surface was modified with an aminosilane prior to the attachment of oligonucleotide. Whereas these functional SAMs can be used to prepare arrays of biomolecules, a bottom-up, layer-by-layer approach is required to avoid potential cross-contamination.

Herein we describe a robust, reliable, and inexpensive method to produce predesigned, spatially controlled, high density microarrays of biomolecules directly on glass (SiO_x) substrates using either μCP or DPN. Our method consists of presilanizing a biomolecule prior to the patterning. A 3'-acrydite-terminated 15-mer oligonucleotide was chosen as the biomolecule test-case because arrayed oligonucleotides have been well studied and can easily be examined for their molecular recognition properties by hybridizing with fluorescently labeled cDNA, thus confirming attachment and retention of biological function.(12)

Materials and Methods

Materials

Mercaptopropyltrimethoxysilane (MPTMS) and all oligonucleotides were purchased from Sigma-Aldrich. (Milwaukee, WI). All chemicals were used as received without further purification. Milli-Q water (18.2 MΩ) was used for all aqueous experiments.

Microcontact Printing of Silanized Oligonucleotides

Oligonucleotides were silanized with MPTMS using 1 mM DNA in Tris/EDTA (10 mM/1 mM) and 5 mM MPTMS in 30 mM NaOAc at pH 4.3. An inking solution was prepared using 30 mM sodium acetate buffer at a pH 4.3 with 10% dimethyl sulfoxide (DMSO). A polydimethylsiloxane (PDMS) stamp was inked by placing a drop of the MPTMS-DNA solution on the stamp face for ∼15 s and drying under dry nitrogen. The dried stamp and the glass (SiO_x) substrate were brought into contact for ∼15 s, after which the SiO_x substrate was left at room temperature to air-dry for ∼15 min. The patterned substrate was then heated to 50 °C for ∼15 min and dipped into boiling water for 30 s to facilitate curing, after which it was rinsed with ethanol and dried with dry nitrogen.

Dip-Pen Nanolithography of Silanized Oligonucleotides

Tween-20 (1%) was added to NaOAc buffer (30 mM sodium acetate buffer at a pH 4.3 with 10% DMSO) to improve diffusion of MPTMS-DNA during the DPN process by making the solvent more wettable.(20) Freshly cleaned SiO_x substrates were used for DPN experiments, and a NanoInk NScriptor system equipped with CAD software was used for all DPN experiments and AFM imaging. Silicon nitride probes with a cantilever spring constant of 0.5 N/m were used. All experiments were performed under ambient conditions, and deposition times varied from 0.1 to 1 s. The samples were cured by air drying at room temperature for ∼15 min, followed by heating at 50 °C for ∼10 min, followed by exposure to boiling water for ∼30 s, and dried with dry nitrogen. DPN-generated MPTMS-DNA patterns were examined with AFM after curing.

Results and Discussion

Several groups have tried to pattern trimethoxysilanes in aqueous solution using μCP without success because of the competing polymerization reaction of silanes and the reaction of the silane ink with the PDMS stamp.(1, 3, 10-13, 20) We hypothesized that these competing polymerization reactions could be limited by keeping the pH below 4.5. To test this hypothesis, a 15-mer oligonucleotide was silanized with MPTMS at pH 4.3 (Figure 1). It should be noted that once oligonucleotides were silanized, they were stable for more than 2 h before significant polymerization was observed. After MPTMS and DNA were coupled, MPTMS-DNA was pattered via μCP. The PDMS stamp was inked by placing a drop of the MPTMS-DNA solution at pH 4.3 on the stamp face and drying under nitrogen. The dried stamp and the SiO_x substrate were brought into contact for ∼60 s, after which the glass (SiO_x) substrate was left at room temperature to air-dry for ∼15 min. Figure 2 shows AFM images of

MPTMS-DNA dot patterns prepared via µCP. Cross-linking between MPTMS and the PDMS stamp surface was minimal, resulting in well-defined µCP patterns. Care must be taken to limit the contact time between the SiO_x surface and the PDMS stamp to prevent the formation of multilayers (as determined by AFM height information as a function of contact time; Figure S1 of the Supporting Information). Thus far, we have observed that ~15 min of curing at a temperature of 50 °C is sufficient when the compound is patterned via µCP. This is in line with the literature, which suggest times ranging from 10 min to overnight at temperatures of 50 to 120 °C.(21, 24)

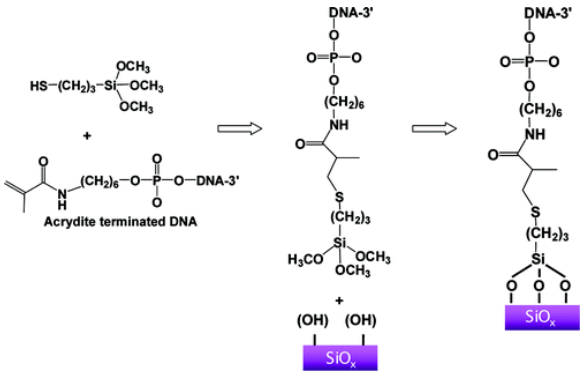

Figure 1. Schematic showing the reaction of acrydite-terminated DNA with MPTMS and the subsequent MPTMS-DNA's reaction with a silicon substrate.

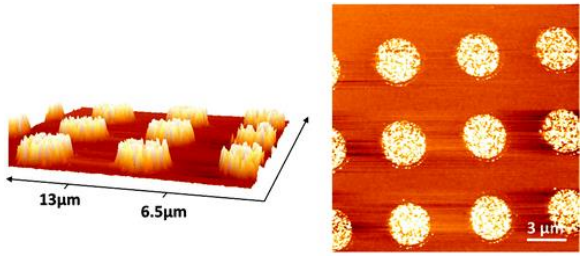

Figure 2. AFM images of MPTMS-DNA dot patterns prepared via µCP.

After patterning, the biological function of the DNA-SiO_x microarray was investigated. The reactivity of the patterned molecules was examined via DNA hybridization using a complementary strand of DNA with a 5'-fluorescent tag (Fluorescein). The printed patterns exhibited a distinct green fluorescence with little or no nonspecific adsorption (Figure 3). The hybridized DNA molecules could be dehybridized by exposing the sample to temperatures at or above the melting point (80 °C). After washing, no fluorescence was observed; however, the surface-bound silanized DNA oligos remained active, as evidenced by rehybridization with complementary 5'-fluorescently tagged DNA strands. Although, the signal intensity is somewhat lower, the substrate remained active after repeating the hybridization/dehybridization cycle more than four times. These data provide "proof-of-concept" that DNA can be directly covalently attached to inert SiO_x surfaces in a predesigned and spatially controlled fashion and retain its biological activity.

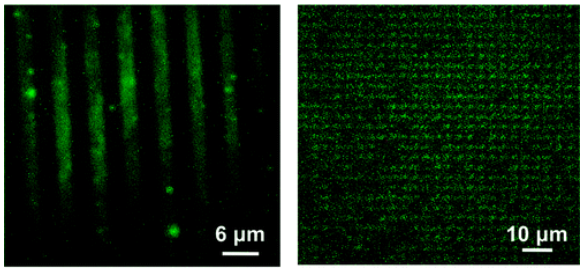

Figure 3. Fluorescence image of the µCP printed patterns of MPTMS-DNA molecules.

Having the ability to prepare biologically active surfaces directly on an inert substrate such as SiO_x via μCP opens the door to preparing a wide range of biomaterials with reproducible, homogeneous spots with high edge resolution. However, μCP may not be the best lithographic choice for patterning biomolecules. For example, the amount of ink transferred to a surface is not well controlled. Moreover, μCP can only reproduce patterns that are predefined by the stamp and cannot generate different patterns in a spatially controlled manner. Therefore, we have also utilized DPN to prepare biologically active surfaces on SiO_x substrates.(25, 26) DPN is a particularly important nanolithographic method for patterning biological molecules because DPN is capable of positioning molecules on a substrate with 10 nm resolution in predesigned, spatially controlled arrays.(25)

Microarrays of MPTMS-DNA were prepared via DPN by direct patterning using a NanoInk NScriptor system with spatial resolution down to a few hundred nanometers. We found that NaOAc buffer (pH 4.3) hinders cross-linking of the silane molecules and the addition of 1% Tween-20 helps with diffusion of MPTMS-DNA during the DPN process by making the solvent more wettable.(20) DPN-generated MPTMS-DNA patterns were examined with AFM after curing in air at room temperature for ∼15 min, followed by air drying at 50 °C for ∼10 min. Typical AFM images of DPN-generated MPTMS-DNA patterns are shown in Figure 4. The estimated height of these DPN-generated MPTMS-DNA patterns measured from randomly placed height profiles using tapping mode AFM (TMAFM) revealed a height of 5.9 ± 0.3 nm, which differs from the height of DPN-generated patterns of just MPTMS by ∼5.0 nm (Figure S2 of the Supporting Information). This difference is consistent with the length of the 15-mer DNA strand used. To ensure that the DNA bound to SiO_x retains its biological activity, it was hybridized with its cDNA strand tagged with a fluorescent probe (Figure 5). The DPN-generated patterns are clearly visible in the images, and the selectivity of the binding is evidenced by little or no nonspecific adsorption. Moreover, these selectively assembled DNA molecules retained their molecular recognition properties through multiple hybridization/dehybridization transformation processes.

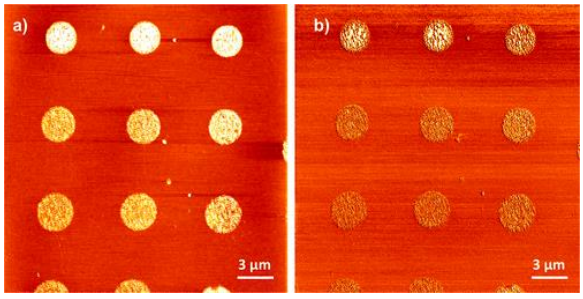

Figure 4. Typical AFM (a) topography and (b) phase images showing DPN-generated dot patterns of MPTMS-DNA molecules.

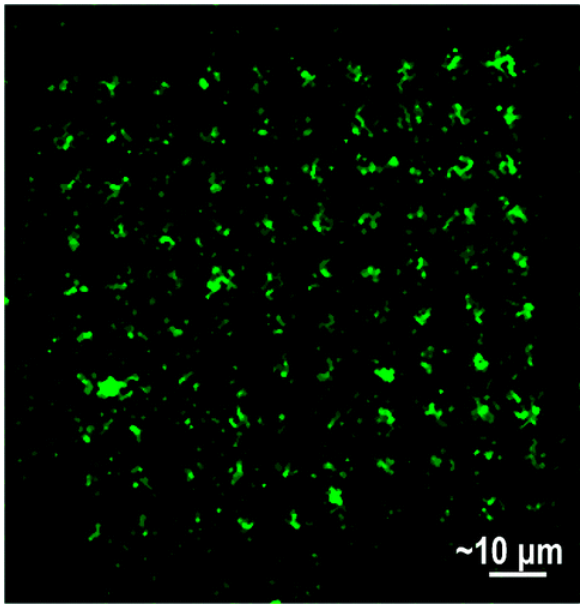

Figure 5. Fluorescence image of DPN patterns of MPTMS-DNA molecules.

In conclusion, the data presented herein provide “proof-of-concept” that silanized-biomolecules such as DNA can be prepared and covalently attached to inert SiO_x surfaces via both µCP and DPN in a predesigned and spatially controlled fashion. Previously, silanes have been patterned on SiO_x surfaces via a bottom-up, layer-by-layer approach, which may result in cross-contamination.(12) Our method allows the direct patterning of a biomolecule on a SiO_x surface and is generally applicable to a wide range of molecules, including those used in light harvesting or catalysis as well as biological molecules such as proteins and antibodies. The ability to pattern silane-functionalized biomolecules on inert or optically transparent semiconductor surfaces will open new research avenues for building complex, multifunctional, and electronically important biological arrays using a very inexpensive and inert substrate.

Supporting Information

AFM topography images and their corresponding height profiles of MPTMS and MPTMS-DNA patterns. This material is available free of charge via the Internet at http://pubs.acs.org.

Terms & Conditions

Acknowledgment

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